challenged by the weak protective activity of mitochondrial Mn-SOD (Sakon et al., 2003; Pham et al., 2004). Putative extramitochondrial sources of TNF-R1-induced ROS have in fact been identified. Ultimately, identifying the origin of ROS will require employing genetic tools and more sophisticated methods for detecting early ROS and discriminating individual species.

Finally, there is evidence to suggest that the coupling of ROS and JNK signaling downstream of TNF-Rs is bidirectional. It has been proposed that in the TNF-R1-triggered pathway for necrosis, ROS lie downstream (rather than upstream) of JNK (Ventura et al., 2004). Thus, the molecular ordering of JNK and ROS signaling might differ depending upon the type of PCD response initiated by TNF-Rs. Indeed, this represents another important issue for future investigation.

The actual outcome of TNF-R stimulation depends upon the biological context and tissue in which this stimulation occurs. Undoubtedly, major future challenges include determining the precise mechanisms by which ROS promote JNK activation and PCD and assessing which target genes are most relevant to the antioxidant activity of NF-κB in specific tissues and contexts. The use of conditional knockout models will be key for addressing these issues. Because the NF-κB-mediated attenuation of TNFα-induced killing plays a crucial role in human diseases, gaining understanding of how ROS trigger PCD and how NF-κB promotes survival might enable development of entirely novel approaches to treatment of these diseases, one that is effective and yet lacks the serious immunosuppressive side effects of general NF-κB blockers.

The study by Kamata et al. provides an exemplary illustration of this concept. It shows that in the liver, ROS-mediated activation of JNK signaling plays a selective role in TNF-R-mediated hepatic injury induced by concanavalin-A, but not in regeneration postpartial hepatectomy, albeit both processes are governed by integration of activities of TNF-Rs, JNK, and NF-κB. Identifying the mechanisms responsible for ROS-mediated JNK induction and NF-κB-dependent protection in patho-physiological contexts such as these represents a major challenge yet holds great promise of yielding the key for a new type of approach to therapy.

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Selected Reading

De Smaele, E., Zazzeroni, F., Papa, S., Nguyen, D.U., Jin, R., Jones, J., Cong, R., and Franzoso, G. (2001). Nature *414*, 308–313.

Kamata, H., Honda, S., Maeda, S., Chang, L., Hirata, H., and Karin, M. (2005). Cell *120*, 649–661.

Matsuzawa, A., and Ichijo, H. (2005). Antioxid. Redox Signal. *7*, 472–481.

Pham, C.G., Bubici, C., Zazzeroni, F., Papa, S., Jones, J., Alvarez, K., Jayawardena, S., De Smaele, E., Cong, R., Beaumont, C., et al. (2004). Cell *119*, 529–542.

Reuther-Madrid, J.Y., Kashatus, D., Chen, S., Li, X., Westwick, J., Davis, R.J., Earp, H.S., Wang, C.Y., and Baldwin, A.S. (2002). Mol. Cell. Biol. *22*, 8175–8183.

Sakon, S., Xue, X., Takekawa, M., Sasazuki, T., Okazaki, T., Kojima, Y., Piao, J.H., Yagita, H., Okumura, K., Doi, T., and Nakano, H. (2003). EMBO J. *22*, 3898–3909.

Tang, G., Minemoto, Y., Dibling, B., Purcell, N.H., Li, Z., Karin, M., and Lin, A. (2001). Nature *414*, 313–317.

Ventura, J.J., Cogswell, P., Flavell, R.A., Baldwin, A.S., and Davis, R.J. (2004). Genes Dev. *18*, 2905–2915.

Wajant, H., Pfizenmaier, K., and Scheurich, P. (2003). Cell Death Differ. *10*, 45–65.

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A New Function for the Elongator Complex: Polarization of Rab Activity?

The Elongator complex was first identified through association with hyperphosphorylated forms of RNA polymerase II and was thought to have a role in transcriptional elongation in yeast. In this issue of *Molecular Cell*, Rahl et al. suggest a novel function for this complex: regulating polarized cell-surface transport. Defects in the human form of this complex result in a neurodegenerative disease, familial dysautomia (FD), suggesting that a deficiency in neuronal polarized trafficking is the underlying cause of FD.

The activation of the Rab GTPase Sec4 is thought to be a key step in polarized cell surface trafficking in yeast. The guanine nucleotide exchange factor Sec2p is responsible for Sec4p activation and is itself localized to sites of polarized growth (Walch-Solimena et al., 1997; Elkind et al., 2000). In order to find regulators of Sec2 function, Rahl et al. (Rahl et al., 2005) initiated a simple screen for mutants capable of suppressing the temperature-sensitive growth of *sec2-59* cells. From this screen, they identified a loss-of-function mutant in

the *ELP1* gene. Previous work had identified Elp1 as a component of a multisubunit complex termed the Elongator complex. Like *elp1Δ*, loss of genes encoding either of two other Elongator subunits, Elp2 or Elp3, resulted in suppression of the *sec2-59* mutant, consistent with the idea that the proteins in the Elongator complex function together in this pathway. Surprisingly, Rahl et al. found that the suppression of the *sec2-59* defect did not appear to be working through an effect on transcription: There were no obvious effects on Sec2 protein levels, mutants in genes known to regulate transcriptional elongation failed to suppress *sec2-59*, and the elongation inhibitor 6-AU also failed to have any effect on *sec2-59*. The most compelling evidence that Elp1p (and by extension the Elongator complex) is not suppressing through transcriptional elongation comes from examination of the subcellular localization of GFP-Elp1p. Elp1p had previously been shown to be primarily localized in the cytoplasm (Pokholok et al., 2002). However, this did not eliminate the possibility that Elp1p/Elongator transiently shuttles through the nucleus as part of its normal intracellular intinerary. Using a clever combination of mutants defective in nuclear export and Elp1-GFP constructs with nuclear localization/nuclear export signals (NLS/NES) added as controls, Rahl et al. make a convincing case that Elp1p is present exclusively in the cytoplasm and does not even transiently reside in the nucleus. This conclusion has important implications not only for the mechanism by which the Elongator complex functions in exocytosis, but also for whether the complex has any direct role in transcriptional elongation in yeast. This function had already been questioned by several recent reports that failed to find evidence for Elongator association with RNA PolII on actively transcribing genes (Pokholok et al., 2002; Krogan et al., 2002). Taken together, these data suggest a radically different view of Elongator function: that its primary cellular role may be directed at cytoplasmic regulatory events. How this new view relates to its previously characterized role in transcriptional elongation remains to be resolved.

How might cytoplasmic Elongator complex work on Rab GTPase-mediated polarized trafficking? The genetic and cell biological data presented by Rahl et al. suggest that the answer to this may be complicated in that it appears that Elongator has both positive and negative aspects of its function within the cell. The positive regulatory component comes from multiple lines of evidence that demonstrate that Elp1p is physically associated with the Rab exchange factor Sec2. Importantly, they find that the site of interaction in Sec2 includes the COOH-terminus, which has previously been shown to be required for the localization of the exchange factor to sites of polarized growth (Elkind et al., 2000). Consistent with this mapping, Rahl et al. find that *elp1Δ*—or an Elp1 mutant lacking the Sec2 binding region—shows a pronounced defect in the polarization of the Sec2-GFP. Moreover, the Elp1 mutant lacking the Sec2p interaction domain was unable to complement *elp1Δ*. Thus, Elp1 appears to have a positive role in promoting the polarization of Sec2p.

On the other hand, the fact that *elp1Δ* and two other elongator subunit mutants, *elp2Δ* and *elp3Δ*, act as recessive suppressors of *sec2-59* strongly suggests that it works as a negative rather than positive regulator of Sec2 function in the cell. This is not through relief of a Sec2p inhibitory function unrelated to its Rab exchange activity because *elp1Δ* was also found to suppress a temperature-sensitive allele of *SEC4*, the downstream Rab target of Sec2p. These data indicate that the Elongator complex serves to downregulate the function of the post-Golgi secretory pathway, perhaps in response to cues that coordinate cell growth with cell-cycle progression (Kozminski et al., 2003).

A particularly interesting set of observations concerns the role of acetyltransferase activity in Elongator function in this pathway. The Elp3 subunit of Elongator has been shown to possess acetyltransferase activity (Wittschieben et al., 1999). Using a mutant form of Elp3 with a point mutation known to abolish acetyltransferase activity, Rahl et al. show that this form of the protein strongly suppresses the *sec2-59* mutant—demonstrating that this enzymatic activity is essential to the negative regulatory function of this complex. Identification of the target(s) of this acetyltransferase function will be critical to delineating the precise mechanism by which Elongator acts in this pathway.

Possible targets for acetylation by the Elongator complex could include a Sec4 GTPase Activating Protein (GAP) or Sec2p—the Sec4 exchange factor. In the

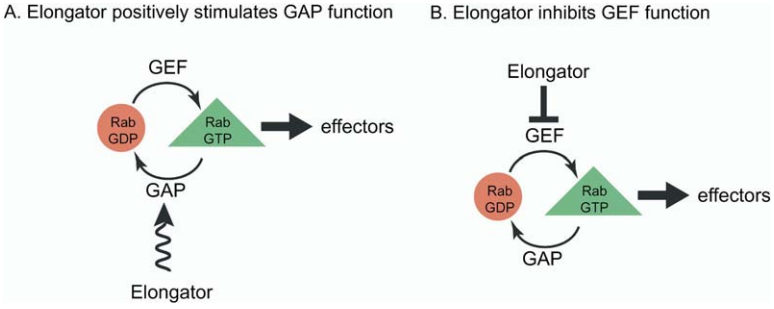

Figure 1. Two Models for Regulation of Rab Activity by Elongator

(A) Elongator acetylation would activate a negative regulator of Rab function, Rab GAP. (B) Elongator acetylation would inhibit a positive regulator of Rab function, Rab GEF. The red circles represent the GDP bound form of the Rab, and the green triangles represent the active GTP bound form of the Rab.

first case (Figure 1A), one would predict that the acetylation of a Sec4 GAP would act in a stimulatory fashion, such that loss of Elongator would result in inhibition of the GAP and thus stabilization of Sec4-GTP. Alternatively, the Sec4 exchange factor itself, Sec2p, could be the target of Elongator regulation. In this model (Figure 1B), modification of Sec2p by Elongator would have an inhibitory effect on Sec2 exchange activity. In this manner, Elongator deletion would result in increased Sec4 exchange activity. Conceivably, the acetyltransferase may act on other components involved in polarity establishment and maintenance in addition to the exocytic apparatus.

Deficiencies in the human homolog of Elp1p, also known as IKAP, are responsible for a neurodegenerative disease called familial dysautonomia (FD). The neuronal specificity of this defect is thought to be due to a tissue-specific splicing defect manifested in neurons (Slaugenhaupt and Gusella, 2002). IKAP is primarily found in a human-cell complex that is strikingly similar to the yeast Elongator complex (Hawkes et al., 2002). This new work by Rahl et al. suggests a radically different model for the function of Elongator in yeast, which if conserved in mammalian cells, would be of great importance in terms of understanding the normal role that these proteins play in polarized trafficking, but also in terms of understanding the underlying defect contributing to familial dysautonomia.

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Selected Reading

Elkind, N.B., Walch-Solimena, C., and Novick, P.J. (2000). J. Cell Biol. *149*, 95–110.

Hawkes, N.A., Otero, G., Winkler, G.S., Marshall, N., Dahmus, M.E., Krappmann, D., Scheidereit, C., Thomas, C.L., Schiavo, G., Erdjument-Bromage, H., et al. (2002). Mol. Cell *3*, 109–188.

Kozminski, K.G., Beven, L., Angerman, E., Tong, A.H., Boone, C., and Park, H.O. (2003). Mol. Biol. Cell *14*, 4958–4970.

Krogan, N.J., Kim, M., Ahn, S., Zohng, G., Kobor, M., Cagney, G., Emili, A., Shilatifard, A., Buratowski, S., and Greenblatt, J.F. (2002). Mol. Cell. Biol. *22*, 6979–6992.

Pokholok, D.K., Hannett, N.M., and Young, R.A. (2002). Mol. Cell *9*, 799–809.

Rahl, P.B., Chen, C.Z., and Collins, R.N. (2005). Mol. Cell *17*, 841–853.

Slaugenhaupt, S.A., and Gusella, J.F. (2002). Curr. Opin. Genet. Dev. *12*, 307–311.

Walch-Solimena, C., Collins, R.N., and Novick, P.J. (1997). J. Cell Biol. *37*, 1495–1509.

Wittschieben, B.O., Otero, G., de Bizemont, T., Fellows, J., Erdjument-Bromage, H., Ohba, R., Li, Y., Allis, C.D., Tempst, P., and Svejstrup, J.Q. (1999). Mol. Cell *4*, 123–128.